PB83-222950

Perturbations in the Biomass, Metabolic Activity, and Community Structure of the Estuarine Detrital Microbiota: Resource Partitioning in Amphipod Grazing

Florida State Univ., Tallahassee

Prepared for

Environmental Research Lab., Gulf Breeze, FL

1982

U.S. Department of Commerce
National Technical Information Service
PB83

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. REC
EPA-600/J-82-324		PB83-222950

4. TITLE AND SUBTITLE
PERTURBATIONS IN THE BIOMASS, METABOLIC ACTIVITY, AND COMMUNITY STRUCTURE OF THE ESTUARINE DETRITAL MICROBIOTA: RESOURCE PARTITIONING IN AMPHIPOD GRAZING (Journal Version)

5. REPORT DATE
1982

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Department of Biological Science
Florida State University
Tallahassee, FL 32306

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.
R806143

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory
Office of Research and Development
Gulf Breeze, FL 32561

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/04

15. SUPPLEMENTARY NOTES
J. Exp. Mar. Biol. Ecol. 1982 64:124-143

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	unclassified	20
	20. SECURITY CLASS (This page)	22. PRICE
	unclassified	

EPA-600/J-82-324
JOURNAL ARTICLE

J. Exp. Mar. Biol. Ecol., 1982, Vol. 64, pp. 125–143
Elsevier Biomedical Press

PERTURBATIONS IN THE BIOMASS, METABOLIC ACTIVITY, AND COMMUNITY STRUCTURE OF THE ESTUARINE DETRITAL MICROBIOTA: RESOURCE PARTITIONING IN AMPHIPOD GRAZING

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Abstract: With the development of quantitative methods for estimating the biomass, metabolic activity, nutritional status and community structure of the estuarine detrital microbiota, and the indication of marked effects on the microbiota by amphipod grazing, a detailed examination of the effects of grazing by two sympatric amphipods was conducted. The microbiota, which develop on Teflon squares after a 2-wk exposure in a North Florida estuary, showed, when grazed for 2 wk, significantly increased bacterial biomass (short-branched and *cis*-vaccenic fatty acids) and microeukaryotes (total polyenoic fatty acids). Grazing in the laboratory at field density further increased the bacterial biomass (muramic acid, wall glucosamine), as well as the total photosynthetic biomass (lipid galactose) if compared to the microbiota grazed in the field. Grazing in the laboratory also depressed the algae estimated as alpha linolenic acid but stimulated the fast growing diatoms (20 carbon polyenoic fatty acids of the alpha linolenic series). The field-grazed microbiota showed metabolic stress indicated by the higher adenosine to adenosine triphosphate (ATP) ratio.

Two sympatric amphipods *Gammarus mucronatus* (Say) and *Melita appendiculata* (Say) having markedly different mouth parts were shown to partition the detrital microbiota. *Melita* feed on nonphotosynthetic microeukaryotes (lowered triglyceride glycerol) leaving a larger total biomass (extractable lipid phosphate), photosynthetic biomass (lipid galactose) with greater total metabolic activity estimated as the 4-h rate of phospholipid and sulfolipid synthesis. Bacterial biomass increased (muramic acid, short-branched and *cis*-vaccenic fatty acids) compared with the detritus grazed by *Gammarus*. *Gammarus* feeds less specifically on bacteria and photosynthetic microeukaryotes. Bacteria (muramic acid) and microeukaryotes (the ratio of lipid glycerol to lipid phosphate) were significantly decreased. This feeding provokes an intense metabolic activity from the lowered biomass that is reflected in the significantly higher total adenosine nucleotides, ATP, and adenylate energy charge. The amphipods from the field and laboratory microcosms showed similar adenylate energy charges and neutral lipid glycerol to phospholipid ratios, indicating their nutritional status was similar.

INTRODUCTION

Detritus forms the basis of many estuarine food webs. Actually it is the detrital microbiota which, by concentrating nutrients from the water column and the detritus itself into a nutritionally adequate biomass, supports the food chain (Melchiorri-Santolini & Hopton, 1972; Fenchel & Jorgensen, 1977; Christian & Wetzel, 1979; Morrison & White, 1980). With newly developed biochemical methods it is possible to define quantitatively the detrital microbiota and with these methods to determine the adequacy of laboratory microcosm experiments in mirroring the field. The composition of the detrital surface markedly affects the biomass and composition of the detrital

0022-0981/82/0000-0000

microbiota which in turn affects the grazing community attracted to the detritus (White *et al.*, 1979d) or the growth of polychaetes (Tenore, 1977). The biodegradability of detritus with similar morphology (needles of *Pinus elliottii* compared to polyvinyl chloride needles) induced a 2- to 10-fold greater microbial biomass with distinctly different enzymatic activities (Bobbie *et al.*, 1978).

One of the most marked effects on the detrital microbiota results from grazing and no study of the detrital microbiota proposing to apply to the real world can ignore the effects of grazers. Hargrave (1976) found bacterial respiration increased to a maximum, then decreased as the density of the benthic deposit-feeding amphipod *Hyalella azteca* was increased. Grazers keep the microbial community from achieving self-limiting numbers (Harrison & Mann, 1975; Fell *et al.*, 1976), fulfilling the prediction of Johannes (1965) that grazing confers perpetual youth on the detrital microbiota.

The amphipods used in the present study were shown by scanning electron micrography to graze estuarine detritus without damaging the delicate physical structures of leaves of *Quercus virginiana* Mill and to increase ^{14}C released from prelabeled detrital microbiota to the water column, as well as increasing the ^{14}C recovered in amphipod feces and specific amphipod tissue components (Morrison & White, 1980). Amphipod grazing increased metabolic activities of the detrital microbiota measured as the rate of oxygen utilization and the rates of neutral-, glyco-, and phospholipid synthesis. The rate of synthesis of the prokaryotic endogenous storage lipid poly beta-hydroxybutyrate and the rates of $^{14}CO_2$ release from prelabeled microbiota were shown to be stimulated by amphipod grazing (Morrison & White, 1980). In the aforementioned study, the microbial metabolism was measured as the increased rate of neutral-, glyco- and phospholipid turnover after "pulse-chase" exposure to ^{14}C labeled acetate and $H_3{}^{32}PO_4$. One lipid component, the glycerophosphorylcholine derived from phosphatidyl choline in the detrital microbiota, showed no turnover in the "pulse-chase" experiments. The loss of the [^{14}C]glycerolphosphorylcholine was a direct measure of the rate of amphipod predation (Morrison & White, 1980). These metabolic activities increased to a maximum and then decreased as the grazing pressure was intensified (Morrison, 1980).

Many detritivores not only graze the microbiota but shred the detritus thereby increasing the surface area for microbial recolonization and enhanced microbial degradation (Odum & De la Cruz, 1967). This has been shown for amphipods (Fenchel, 1970; Harrison, 1977; Lopez *et al.*, 1977), gastropods (Fenchel, 1972, 1977), and combinations of polychaetes and nematodes (Lee *et al.*, 1975; Tenore *et al.*, 1977).

The surface topography of sedimentary particles can affect the microbial biomass and community structure. In the recolonization of sand in running sea water, the presence of microdiscontinuities greatly increased the biomass of both bacteria and microflora possibly as a response to grazing pressure (Nickels *et al.*, 1981).

Selectivity in grazing the microbiota has been correlated with the morphology of the mouthparts of nematodes as reviewed by Twinn (1972) and in deposit-feeding amphipods and gastropods (Fenchel *et al.*, 1975). Four sympatric haustoriid amphipods

showed different mouthpart morphologies (Ivester & Coull, 1975) and these differences were proposed as important isolating mechanisms in allowing fractionation of food resources. Amphipod grazing will be shown to affect markedly the biomass, metabolic activities, nutritional status, and community structure of the detrital microbiota. The objectives of this work are to utilize biochemical markers for biomass, metabolic activity, and community structure of the surface microbiota to define: (1) the effect of amphipod grazing, (2) the differences in the effects of grazing in the field and laboratory, and (3) to compare the grazing of *Melita appendiculata* (Say) to *Gammarus mucronatus* (Say), two sympatric amphipods with different mouth parts.

METHODS

MATERIALS

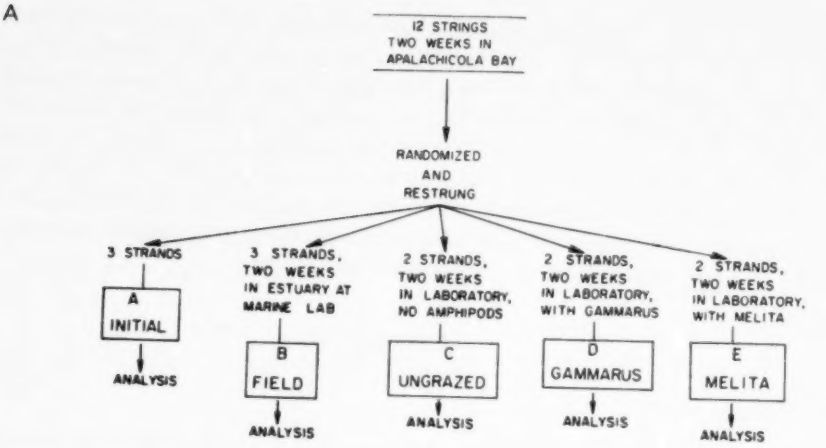
Nanograde or glass distilled solvents (Burdick and Jackson, Muskegon, MI, U.S.A.) and freshly redistilled chloroform were utilized. Standards and derivatization reagents were purchased from Supelco (Bellefonte, PA, U.S.A.); Applied Science Co. (State College, PA, U.S.A.); Aldrich, Inc. (Milwaukee, WI, U.S.A.); Sigma Chemical Co. (St. Louis, MO, U.S.A.), Pierce Chemical Co. (Rockford, IL, U.S.A.); and PCR Research (Gainesville, FL, U.S.A.). [^{3}H]thymidine, $H_3{}^{32}PO_4$, and $H_2{}^{35}SO_4$ were supplied by New England Nuclear Corp. (Boston, MA, U.S.A.).

DETRITAL SAMPLES AND AMPHIPOD EXPOSURE

Teflon sheets 5 μm thick were cut into 5-cm^2 squares and strung on nylon monofilament lines in groups of 30 in such a way that each surface was equally exposed to the water. These strings of squares were placed in baskets made of neoprene coated 2.5-cm mesh hardware cloth 30 cm on a side and incubated in Apalachicola Bay in the northern Gulf of Mexico (29°43.64′N : 85°57.6′W) for 2 wk in the spring of 1980 at a depth of 1 m (White *et al.*, 1979c). The baskets were then transferred in aerated estuarine water to the Florida State University Marine Laboratory 72 km to the east. The strings of Teflon squares were cleared of visible invertebrates, randomized and restrung on monofilament lines. At the marine laboratory, sets of two strands of 30 squares were suspended in each of three 15-l aquaria through which settled but unfiltered sea water flowed at 400 ml · min^{-1} (95% turnover · day^{-1}) (salinity, 26.5 ± 2‰; dissolved oxygen, 7.8 + 0.2 μg · l^{-1}; temperature, 16 ± 2°C; pH, 8.1 ± 0.08; ambient light and photoperiod). Three strands of Teflon leaf strings were returned to baskets placed just outside the marine laboratory (29°54.5′N : 84°31′W).

After an exposure of an additional 2 wk in the field, or in the laboratory under various grazing pressure, the biomass, community structure and metabolic activities were analyzed (Fig. 1). The Teflon squares were removed from the strings into a beaker containing sea water mixed thoroughly and randomly selected for analysis.

A



		NUMBER OF AMPHIPODS / 1500 cm²		
GAMMARUS	18	125	0	300	0
MELITA	1	60	0	0	300
COROPHIUM	3	138	0	0	0

B

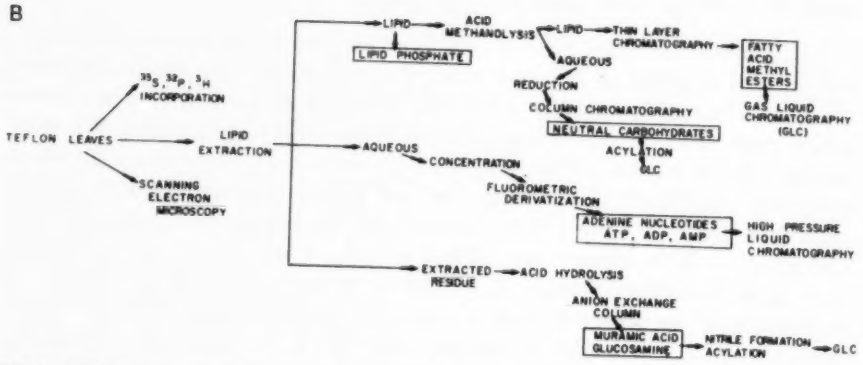


Fig. 1. A, diagram of the exposure of Teflon squares for inoculation and subsequent treatments with exposure to amphipod grazing in the field and laboratory or protection from amphipod grazing: amphipod densities per 1500 cm^2 of Teflon surface are given below each treatment; B, flow diagram of the biochemical analysis of the estuarine surface microbiota exposed as in A.

AMPHIPODS

Amphipods were recovered from the field stations. The sex and size distributions were not altered from the proportions in the field. *Gammarus* (*G. mucronatus* and *G. macromucronatus* in a 2 : 1 ratio); *Melita* (*M. appendiculata* 90% with no *Gammarus*); and *Corophium* (*C. tuberculatum*, *C. louisianum* in a 4 : 1 ratio) were recovered and utilized as indicated in Fig. 1A. The surface microbiota was exposed to all three amphipods at a density of 0.22 amphipods $\cdot$ cm^{-2} in the field or to either *Gammarus* or *Melita* at a density of 0.2 amphipods $\cdot$ cm^{-2} in the laboratory. *Gammarus mucronatus* and *Melita appendiculata* make up the major macroscopic detrital grazers found in the northeast coast of the Gulf of Mexico (Livingston *et al.*, 1977).

ANALYSIS

A flow diagram of the analysis of the surface microbiota is given in Fig. 1B. The lipids were extracted with a modified one phase Bligh & Dyer (1959) extraction (White *et al.*, 1979b). The chloroform phase was recovered, filtered through Whatman 2V fluted filter paper, reduced in volume in a stream of nitrogen, and a portion utilized for the colorimetric determination of the lipid phosphate after digestion (White *et al.*, 1979b). The major portion was subjected to mild acid methanolysis and the lipid components from this procedure fractionated by thin layer chromatography and analyzed by glass capillary gas-liquid chromatography (GLC) (Bobbie & White, 1980). Authentication of the assignments of the lipid fragments was by gas chromatography–mass fragmentation spectrometry using the Hewlett-Packard 5995A GC/MS. Lipid carbohydrates and neutral lipid glycerol were determined from the aqueous portion of the methanolysis partitioning, reduced and peracetylated prior to analysis by GLC (Bobbie *et al.*, 1981).

The aqueous phase of a lipid extraction designed for maximum recovery of adenosine nucleotides was concentrated, derivatized with chloroacetaldehyde, separated by high pressure liquid chromatography, and detected by fluorescence spectroscopy (Davis & White, 1980). The organic phase of the extraction was added to the lipid extractions described above.

The residue after lipid extraction was subjected to acid hydrolysis, purification by column chromatography, aldonitrile formation, peracetylation and analysis by GLC (Fazio *et al.*, 1979; Findlay *et al.*, 1981).

Determination of the rates of synthesis was performed on triplicate sets of five randomly selected Teflon squares incubated in the dark with 400 μCi [^{3}H]thymidine (20 mCi $\cdot$ mmol^{-1}), 100 μCi $H_3{}^{32}PO_4$ (320 mCi $\cdot$ mmol^{-1}) of 160 μCi $H_2{}^{35}SO_4$ (4.3 mCi $\cdot$ μmol^{-1}) in 300 ml of filter sterilized sea water for 4 h. The leaves incubated with ^{32}P and ^{35}S were extracted, the chloroform phase evaporated to dryness, and the radioactivity determined by scintillation spectrometry (King *et al.*, 1977). The incorporation of [^{3}H]thymidine into DNA was determined after extraction with mild alkali (Tobin & Anthony, 1978; White *et al.*, 1980a).

Teflon squares and amphipods were removed from the experiments, fixed with

glutaraldehyde, critical point dried, coated and examined with the Cambridge Stereoscan S4-10 microscope (Cambridge Instrument Co., Ossining, NY, U.S.A.) as described in Morrison *et al.* (1977), except that filter sterilized estuarine water was substituted for the buffer.

Fatty acids are designated as the number of carbon atoms: the number of double bonds with w indicating the position of the double bond nearest the end of the carbon chain opposite the carboxyl group. The prefixes a, i, and delta indicate anteiso, iso, and cyclopropane branching. ATP, ADP, and AMP indicate adenosine triphosphate, adenosine diphosphate and adenosine monophosphate.

STATISTICAL ANALYSIS

An analysis of variance using a completely randomized one factor design with five treatments was tested for significance at alpha = 0.05 using program 26-1705 with a Radio Shack TRS-80 computer. Tukey's wholly significant difference test (WSD) (Meyers, 1979) was utilized to determine significant differences between treatment means while maintaining the error rate per family at alpha = 0.05.

RESULTS

MORPHOLOGIC EFFECTS OF AMPHIPOD GRAZING

Scanning electron microscopic examination of the estuarine surface microbiota after exposure to amphipod grazing in the field or laboratory showed a general diminution in the biomass and morphologic diversity of the microbial assemblage when compared to the ungrazed control (Fig. 2A). After exposure to *Melita appendiculata* in the laboratory, a considerably denser surface microbiota (Fig. 2C) remained than was found after grazing by *Gammarus mucronatus* and *G. macromucronatus* in the laboratory (Fig. 2D) or by a *Gammarus*-dominated mixed amphipod population in the field (Fig. 2B). The morphology revealed by scanning electron microscopy can give suggestive indications of resource partitioning, but quantitative estimates of the effects of grazing or resource partitioning require a different analytical procedure. The data to follow will document the fact that the impressions from scanning electron micrographs are not quantitative.

QUANTITATIVE ANALYSIS OF THE EFFECTS OF AMPHIPOD GRAZING

Teflon squares that were incubated in the estuary for 2 wk, developed a microbiota whose composition is indicated in column A in Tables I–IV. Portions of these samples were incubated in the field for an additional 2 wk (column B), or incubated in the laboratory with three treatments (Fig. 1A).

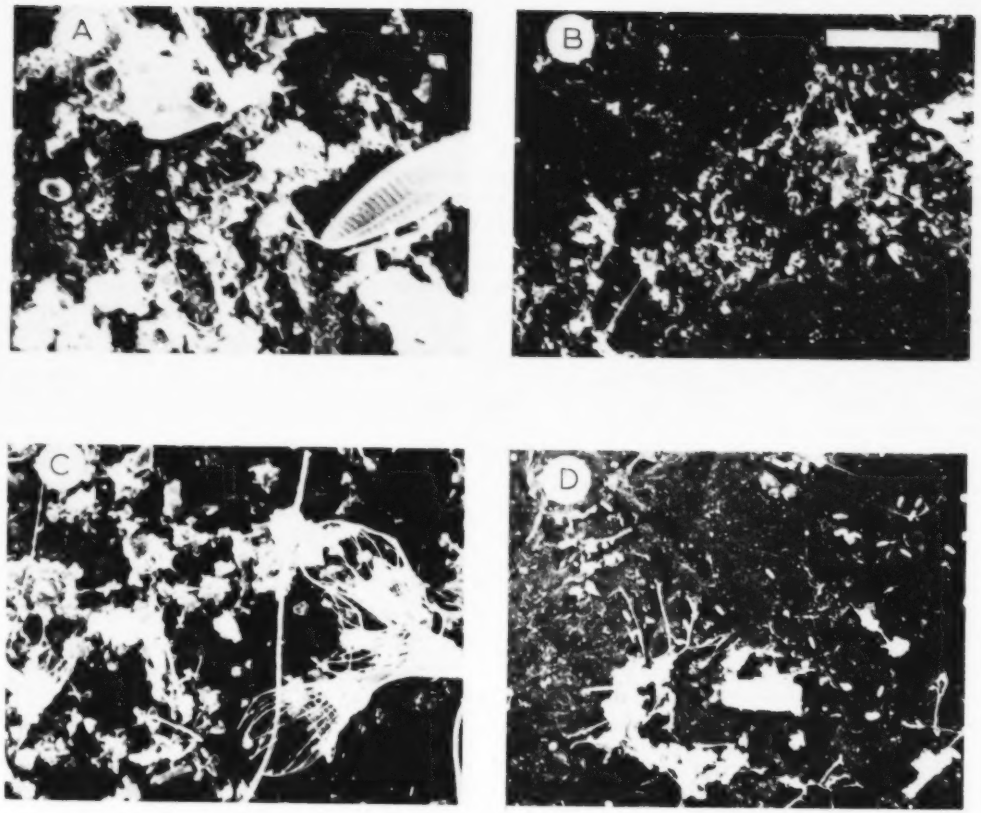


Fig. 2. Scanning electron micrographic morphology of the estuarine surface microbiota on Teflon surfaces after exposure in Apalachicola Bay for 2 wk and then incubation in flowing sea water for 2 wk in the absence of grazing (A), exposed in the field to amphipod grazing with a mixture of amphipods (Fig. 1A) at a density of 0.22 amphipods · cm^{-2} for 2 wk (B), to *Melita appendiculata* in the laboratory at a density of 0.2 amphipods · cm^{-2} (C), or *Gammarus mucronatus* at a density of 0.2 amphipods · cm^{-2} (D): scale bar, 10 μm.

TABLE I

Biomass measures of the estuarine detrital microbiota grazed by amphipods in the field and laboratory.

Component	A Initial	B Field grazed	C Ungrazed	D *Gammarus* grazed	E *Melita* grazed
Lipid phosphate[a]	101 (30.7)	189 (6.2)	95.3 (11.4)	182 (28)	231 (64)
Total palmitate	31.5 (6.5)	41.1 (18)	61.2 (27.6)	60.9 (5.4)	68.3 (7)
Lipid glycerol	4.4 (1.4)	30.0 (11)	38.0 (15)	92.0 (20)	21.0 (11)
Lipid galactose	32.0 (7)	23.0 (20)	12.0 (4)	41.0 (35)	46.0 (29)
Muramic acid	1.75 (0.1)	0.42 (0.15)	0.46 (0.17)	1.28 (0.14)	2.60 (0.35)
Wall glucosamine	0.20 (0.14)	2.50 (2.40)	1.56 (0.8)	5.54 (1.8)	5.50 (2.0)
Lipid phosphate/palmitate	3.17 (0.34)	5.40 (2.70)	1.80 (0.88)	2.98 (0.21)	3.36 (0.03)
Lipid glycerol/lipid phosphate	0.04 (0.00)	0.08 (0.03)	0.42 (0.2)	0.52 (0.2)	0.09 (0.05)
Lipid phosphate/muramic acid	58.0 (20)	237 (125)	481 (230)	180 (41)	156 (17)
Lipid glycerol/lipid galactose	0.17 (0.06)	1.82 (0.93)	3.70 (2.0)	3.30 (2.2)	0.74 (0.7)
Lipid phosphate/lipid galactose	3.50 (0.5)	2.24 (0.3)	14.8 (9.7)	8.20 (1.8)	3.20 (0.00)
Total adenosine nucleotides[b]	3.49 (0.54)	5.34 (0.44)	5.68 (0.45)	5.98 (0.69)	4.30 (1.31)
^{32}P incorporation into lipid[c]			102 (8)	92 (7.3)	227 (38)
^{35}S incorporation into lipid			18.9 (15)	17.0 (13)	42 (21)
[^{3}H]thymidine into DNA			7.7 (2.8)	14 (4.7)	13 (5.3)

Column A. Teflon leaves removed from the field after a 2-wk incubation, randomized and analyzed; data given as X (SD), $n = 4$.
Column B. Teflon leaves incubated an additional 2 wk in the field exposed to a mixed amphipod population plus unknown grazers. Amphipod density 0.22 animals · cm^{-2}.
Column C. Teflon leaves incubated in running sea water for an additional 2 wk in the absence of amphipod grazers.
Column D. Teflon leaves incubated in running sea water for an additional 2 wk in the presence of *Gammarus* at a density of 0.2 amphipods · cm^{-2}.
Column E. Teflon leaves incubated in running sea water for an additional 2 wk in the presence of *Melita* at a density of 0.2 amphipods · cm^{-2}.
[a] Data given in nmol · 50 cm^{-2} Teflon leaf surface area.
[b] Data given in pmol · 50 cm^{-2} Teflon leaf surface area.
[c] Data given as CPM × 10^{3} · 4 h^{-1} · 700 cm^{-2} Teflon leaf surface area.

TABLE II

Fatty acid concentration and composition in the estuarine detrital microbiota grazed by amphipods in the laboratory and field.

Component	A Initial	B Field grazed	C Ungrazed	D *Gammarus* grazed	E *Melita* grazed
a + i 15 : 0[a]	1.70 (0.1)	2.7 (1.3)	3.3 (1.5)	3.0 (0.9)	4.8 (0.9)
15 : 0	3.6 (0.4)	4.1 (2.1)	5.6 (2.2)	4.2 (0.4)	5.3 (0.3)
delta 17 : 0	0.2 (0.1)	0.3 (0.3)	0.7 (0.3)	0.2 (0.3)	0.6 (0.5)
18 : 1w7	2.0 (0.8)	7.4 (1.6)	7.4 (1.7)	7.3 (1.7)	11.8 (1.7)
18 : 2w6	2.6 (0.2)	4.8 (0.6)	3.9 (1.7)	5.3 (2.6)	6.7 (1.1)
18 : 3w3	0.7 (0.1)	3.1 (0.3)	0.9 (0.3)	0.9 (0.4)	1.3 (0.1)
18 : 3w6	1.1 (1.0)	0.4 (0.1)	0.6 (0.3)	0.8 (0.1)	1.2 (0.3)
20 : 3w6	0.0 (0.0)	0.3 (0.3)	0.1 (0.2)	0.1 (0.2)	0.2 (0.2)
20 : 4w6	0.4 (0.1)	2.1 (0.8)	1.5 (0.4)	2.1 (0.8)	3.7 (0.2)
20 : 5w3	0.0 (0.0)	0.3 (0.1)	0.1 (0.2)	0.3 (0.1)	0.2 (0.1)
22 : 6w3	0.5 (0.3)	1.7 (0.5)	0.5 (0.5)	0.4 (0.4)	0.2 (0.1)
Total polyenoics	4.60 (2.60)	14.6 (3.0)	10.6 (5.30)	20.9 (6.40)	27.7 (3.40)
Polyenoic w3/ w6	0.71 (0.34)	2.17 (0.59)	1.06 (0.18)	2.16 (0.67)	1.50 (0.51)
a + i 15 : 0/ 15 : 0	0.48 (0.08)	0.69 (0.06)	0.60 (0.06)	0.7 (0.16)	0.90 (0.11)
a + i 15 : 0/ 16 : 0	0.05 (0.01)	0.06 (0.00)	0.05 (0.01)	0.05 (0.01)	0.07 (0.02)
delta 17 : 0/ 16 : 0	0.007 (0.001)	0.01 (0.01)	0.01 (0.00)	0.003 (0.005)	0.009 (0.008)
18 : 1w7/ 16 : 0	0.07 (0.04)	0.21 (0.06)	0.14 (0.03)	0.14 (0.01)	0.19 (0.01)
18 : 1w7/ 18 : 1w9	0.24 (0.13)	0.59 (0.12)	0.35 (0.17)	0.41 (0.14)	0.51 (0.05)
18 : 2w6/ 16 : 0	0.09 (0.01)	0.14 (0.06)	0.09 (0.01)	0.10 (0.04)	0.11 (0.01)
18 : 3w3/ 18 : 3w6	3.18 (0.39)	8.75 (2.49)	1.65 (0.99)	1.31 (0.21)	1.08 (0.19)
22 : 6w3/ 16 : 0	0.02 (0.02)	0.05 (0.01)	0.02 (0.00)	0.01 (0.00)	0.02 (0.01)
20 : 4w6/ 16 : 0	0.14 (0.01)	0.06 (0.02)	0.03 (0.01)	0.05 (0.05)	0.06 (0.01)
20 : 5w3/ 16 : 0	0.07 (0.04)	0.21 (0.07)	0.12 (0.02)	0.32 (0.05)	0.33 (0.03)
Total polyenoic > 20w3/ 16 : 0	6.63 (1.10)	4.05 (1.09)	3.64 (0.78)	6.69 (0.78)	5.28 (0.43)
Total polyenoic > 20w6/ 16 : 0	0.14 (0.01)	0.07 (0.02)	0.04 (0.01)	0.05 (0.03)	0.07 (0.07)
Total polyenoic > 20/ 16 : 0	0.11 (0.07)	0.35 (0.10)	0.17 (0.04)	0.38 (0.06)	0.41 (0.05)

Columns A, B, C, D and E as in Table I.

[a] Data given as nmol · 50 cm^{-2} Teflon leaf surface as X (SD), $n = 3$.

TABLE III

Adenosine nucleotide concentrations in the estuarine detrital microbiota grazed by amphipods in the laboratory, field, and in the amphipods themselves.

Component	A Initial	B Field grazed	C Ungrazed	D *Gammarus* grazed	E *Melita* grazed	F *Gammarus*	G *Melita*
Adenosine	1.77 (0.14)	2.46 (0.49)	2.77 (0.06)	2.15 (0.15)	1.47 (0.51)	34.5 (14.5)	34.7 (8.82)
AMP	0.22 (0.07)	0.56 (0.13)	0.55 (0.06)	0.3 (0.05)	0.37 (0.11)	6.6 (4.36)	17.1 (5.26)
ADP	0.34 (0.05)	0.6 (0.15)	0.92 (0.16)	0.69 (0.17)	0.67 (0.26)	47.1 (10.1)	38.6 (4.5)
ATP	1.16 (0.21)	1.72 (0.34)	1.75 (0.19)	2.87 (0.34)	1.77 (0.46)	187 (51)	129 (131)
Energy charge	0.79 (0.07)	0.7 (0.03)	0.68 (0.01)	0.83 (0.01)	0.75 (0.02)	0.88 (0.001)	0.81 (0.03)
ATP/adenosine	0.65 (0.01)	0.74 (0.26)	0.71 (0.06)	1.36 (0.08)	1.23 (0.23)	5.63 (0.94)	3.88 (1.09)

Columns A, B, C, D and E as in Table I, column F and G are of the amphipods themselves.
Data in pmol · 50 cm^{-2} Teflon leaf surface, columns A through E as X (SD), $n = 5$.
Data in pmol · $amphipod^{-1}$ columns F and G.

TABLE IV

Significant difference map (Tukey's wholly significant difference test) of components from Tables I, II, and III showing significantly ($P > 0.05$) greater between sample than within sample differences: values increase from smallest to largest left to right and are significantly different if not joined by a line; A is the initial microbiota, B the field grazed, C the ungrazed, D the *Gammarus* grazed, and E the *Melita* grazed as in Table I.

Component	Lowest		→		Highest
Lipid phosphate	C	A	D	B	E
Lipid glycerol	A	E	B	C	D
Lipid galactose	C	B	A	D	E
Muramic acid	B	C	D	A	E
Wall glucosamine	A	C	B	E	D
Lipid glycerol/lipid phosphate	A	E	B	C	D
Lipid phosphate/muramic acid	A	E	D	B	C
Lipid glycerol/lipid galactose	A	E	B	D	C
Lipid phosphate/lipid galactose	B	E	A	D	C
Total adenosine nucleotides	A	E	B	C	D
^{32}P incorporation	D	C	E		
^{35}S incorporation	D	C	E		
15 : 0	A	B	D	E	C
18 : 1w7	A	D	B	C	E
18 : 3w3	A	C	D	E	B
20 : 4w6	A	C	B	D	E
20 : 5w3	A	C	B	D	E
Total polyenoic > 20	A	C	B	D	E
Total polyenoic > 20 w3/w6	D	A	E	B	C
i + a 15 : 0/ 15 : 0	A	B	C	D	E
18 : 1w7/ 16 : 0	A	D	C	E	B
18 : 3w3/ 16 : 0	D	C	E	A	B
20 : 4w6/ 16 : 0	A	C	D	E	B
20 : 5w3/ 16 : 0	A	C	B	D	E
22 : 6w3/ 16 : 0	D	C	E	A	B
Total polyenoic > 20/ 16 : 0	A	C	B	D	E
Adenosine	E	A	D	B	C
AMP	A	D	E	C	B
ATP	A	B	C	E	D
Energy charge	C	B	E	A	D
ATP/adenosine	A	C	B	E	D

A, B, C, D and E indicate the treatments defined in Table I and are arranged in increasing magnitude from left to right. The lines indicate groups whose means do not differ significantly ($P > 0.05$) but do differ significantly from the groups not within the lines.

EFFECT OF AN ADDITIONAL 2 WK OF INCUBATION ON THE DETRITAL MICROBIOTA

The maps indicating statistical differences between means given in Table IV are set down from smallest to largest left to right. For nearly every parameter the additional 2-wk incubation results in an increase in its value. Significant increases in the fatty acids characteristic of bacteria (15 : 0, 18 : 1w7), decreased ratio of lipid glycerol to lipid galactose are seen (Tables I–IV, columns A vs. B, C, D, E). The influence of grazing in the field and laboratory in the additional 2-wk period results in significantly higher total polyenoic fatty acids longer than 20 carbon atoms, and the proportion of the total polyenoic fatty acids, 20 : 4w6 and 20 : 5w3 relative to 16 : 0 (Tables I–IV, columns A vs. B, D, E). This additional 2 wk of incubation in the absence of amphipod grazing resulted in lower adenylate energy charge and muramic acid as well as lower ratio of lipid glycerol to lipid galactose. The absence of amphipod grazing also resulted in increased adenosine, AMP, total adenosine nucleotide content and ratio of polyenoic fatty acids longer than 20 carbon atoms with w 6 unsaturation (Tables I–IV, columns A vs. C).

EFFECTS OF AMPHIPOD GRAZING

Comparison of the data in Tables I–IV, columns C vs. B, D, E shows significant increases in the proportions of the total polyenoic fatty acids longer than 20 carbon atoms and 20 : 5w3 compared with the palmitic acid in the surface microbiota with grazing.

Comparison of grazing effects upon the surface microbiota in the field to the ungrazed surface microbiota (Tables I–IV, columns B vs. C), shows increases in the ratio of lipid glycerol to lipid phosphate and the total adenosine nucleotide in the ungrazed microbiota and increased muramic acid, 18 : 1w7, 18 : 3w3, and ratios of 18 : 3w3, 20 : 4w6, 20 : 5w3, 22 : 6w3 and total polyenoic fatty acids longer than 20 carbon atoms relative to 16 : 0 in field grazed microbiota.

Comparison of the effects of grazing in the laboratory to the ungrazed surface microbiota (Tables I–IV, column C vs. D, E) shows increases in the ATP/adenosine ratio, adenylate energy charge, muramic acid, lipid galactose, wall glucosamine, 20 : 5w3, total polyenoic fatty acids longer than 20 carbon atoms and the ratios of 20 : 5w3 and total polyenoic fatty acids to 16 : 0.

DIFFERENCES IN THE EFFECTS OF AMPHIPOD GRAZING IN THE LABORATORY AND FIELD

The data of Tables I–IV, columns B vs. D, E show statistically significant increases in the muramic acid, lipid galactose, wall glucosamine, 20 : 5w3, and ATP/adenosine in the laboratory-grazed microbiota compared to the field-grazed microbiota. The field-grazed surface microbiota showed significantly higher 18 : 3w3 and ratios of 18 : 3w3 and 22 : 6w3 to the 16 : 0.

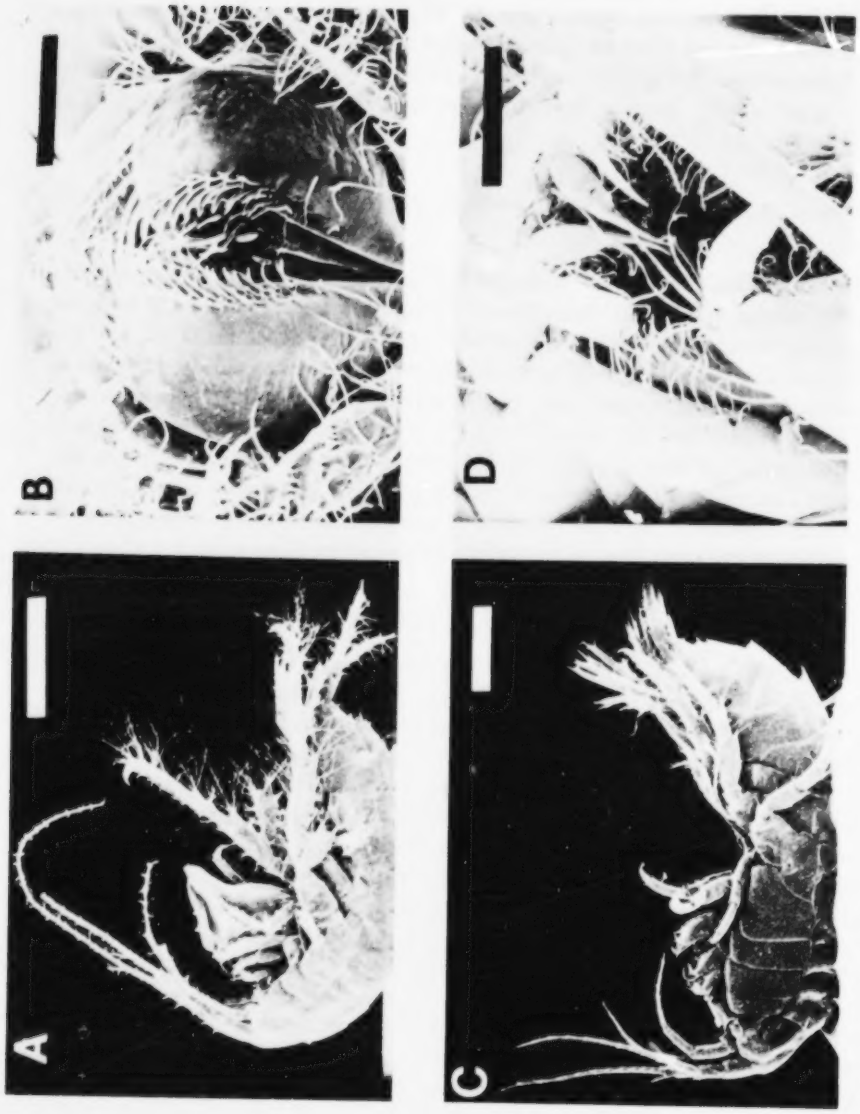


Fig. 3. Scanning electron micrography showing A, the side view (scale bar, 1 mm) and B, mouth parts (scale bar, 100 μm) of *Melita appendiculata*; C, the side view (scale bar, 500 μm), and D, mouthparts (scale bar, 100 μm) of *Gammarus mucronatus*.

MORPHOLOGY OF THE AMPHIPOD FEEDING APPARATUS

Examination of the mouthparts of these two amphipod species shows each might utilize a different feeding method (Fig. 3). The *Gammarus* appears to be a less specialized feeder and might be expected to feed less selectively on the detrital microbiota (Zimmerman, 1979). The morphology of the surface microbiota after grazing by *Melita* shows a richer diversity than after exposure to *Gammarus* in the laboratory or the field (Fig. 2).

DIFFERENCES IN THE DETRITAL MICROBIOTA AFTER GRAZING BY *GAMMARUS* AND *MELITA*

The morphologic differences in the surface microbiota detected in the scanning electron micrographs (Fig. 2) are reflected in the biochemical measures of biomass, community structure and metabolic activity (Tables II–V, columns D vs. E). The microbiota after a 2-wk exposure to grazing by *Melita* showed significant increases in AMP, lipid phosphate, muramic acid, lipid galactose, rates of ^{32}P and ^{35}S incorporation into lipids, 18 : 1w7 and the ratios of i + a 15 : 0/15 : 0 and 18 : 1w7/16 : 0 compared with the surface microbiota grazed by *Gammarus*. Significant decreases in the biochemical components of the detrital microbiota grazed by *Melita* were in lipid glycerol, ratio of lipid glycerol to lipid phosphate, total adenosine nucleotides, adenosine, ATP, and energy charge compared to the microbiota grazed by *Gammarus*.

EFFECT OF LABORATORY CONFINEMENT ON THE AMPHIPODS

In a realistic appraisal of the effects of amphipod grazing on the detrital microbiota in nature, it is critical to be assured that the amphipods in the laboratory are not physiologically stressed. The adenosine nucleotide energy charge of both species in the laboratory was above 0.8 indicating a healthy status (Table III, columns F, G). The ratio of ATP/adenosine is an even more sensitive indicator of metabolic stress (Davis & White, 1980) and is also high in these animals. Preliminary data show that these values are similar to those of amphipods recovered from the field.

Starvation is often reflected in a loss of triglyceride. Triglyceride can be estimated as the neutral lipid glycerol. The total phospholipids representing the membrane biomass are relatively stable during starvation. The lipid phosphate in starved, field-fed and laboratory-fed amphipods was 8.3 (4.8), 7.0 (0.57), and 7.5 (2.8) nmol · amphipod^{-1}($\bar{X}$ (SD), n = 5, measured on 30 amphipods · test^{-1}). These differences are not statistically significant ($P < 0.05$). The neutral lipid glycerol of starved, field-fed, and laboratory-fed amphipods was 0.34 (0.2), 20.7 (1.4), and 14.3 (0.75) nmol · amphipod^{-1}. The fed amphipods have significantly more neutral lipid glycerol than the starved animals. The ratios of neutral lipid glycerol to lipid phosphate for starved, field-fed, and laboratory-fed amphipods were 0.04 (0.00), 2.8 (0.02) and 1.9 (0.7). The ratio of neutral lipid glycerol to phospholipid was significantly greater in the fed animals than the starved

amphipods, but there was no significant difference between the field-fed and laboratory-fed animals.

Discussion

MICROBIAL BIOMASS AND COMMUNITY STRUCTURE INDICATORS

From work with estuarine detritus (Bobbie & White, 1980; Davis & White, 1980; White *et al.*, 1980a; Bobbie *et al.*, 1981) and marine sediments (White *et al.*, 1979a,b; 1980b), it has become possible to associate certain biochemical measurements with relatively specific components of the microbiota. The prokaryotic bacterial component contains muramic acid; acid hydrolyzable wall glucosamine; most of the fatty acids a + i 15 : 0, 15 : 0, delta 17 : 0 and 19 : 0; 18 : 1w7; the greatest concentration (per g dry wt) of phospholipids and total adenosine nucleotides; and the activities measured by the incorporation of [^{3}H]thymidine into DNA and much of the phospholipid synthesis. The microeukaryotes contain the triglyceride glycerol, the lipid inositol, the polyenoic fatty acids longer than 20 carbon atoms, and the monohydroxy steroids. The photosynthetic microeukaryotes (microflora) contain the lipid galactose, the sulfolipid synthetic activity, and the polyenoic fatty acids of the alpha (w3) linolenic acid series. Diatoms are particularly enriched in 16 and 20 carbon polyenoic fatty acids of the alpha linolenic series (w3) (Darley, 1977; Volkman & Johns, 1977). The microfauna contain the polyenoic fatty acids of the gamma (w6) linolenic acid series (Erwin, 1973). Validation of these assignments has come by analyzing mixtures of monocultures; by isolating organisms from their environment and finding "signature lipids"; by manipulating communities with nutrients, light, and specific inhibitors; and by examining the effects of grazing by predators of established specificity.

The nutritional status of the microbiota or their grazers can be determined biochemically. The short-term response to stress can be measured by the adenylate energy charge. The adenylate energy charge is a sensitive measure of the nutritional status of living cells (Knowles, 1971). An even more sensitive indicator of stress is a measure of one of the homeostatic mechanisms by which the cells can compensate for the stress-induced decreased synthesis of ATP. This method involves depressing the AMP level in parallel to the decreased ATP formation thereby maintaining the energy charge. One mechanism for depressing the AMP results in the formation of intercellular adenosine, and the ATP/adenosine ratio has proved a very sensitive indicator of stress (Davis & White, 1980). On a long-term basis, the depression of triglyceride stores with starvation (measured as the neutral lipid glycerol) has proved a measure of the nutritional status of the microeukaryotes (Gehron & White, 1981).

With these data in hand, it is possible to examine the effects of amphipod grazing on the biomass, metabolic activity, and community structure of the estuarine detrital microbiota.

EFFECTS OF GRAZING ON MICROBIAL SUCCESSION

The additional 2-wk exposure in both the laboratory and the field in the presence of grazing amphipods induces significant increases in the bacteria (15 : 0 and 18 : 1w7 fatty acids) and greater increases in the microeukaryote community (total polyenoic fatty acids longer than 20 carbon atoms and the proportions of 20 : 4w6 and 20 : 5w3 to 16 : 0). These findings reflect the previous morphologic and biochemical observations of a bacterial enriched pioneering microbiota that is replaced by a morphologically complex community of both microeukaryotes and bacteria on natural substrates incubated in the field and exposed to grazing (Morrison *et al.*, 1977), but would not be expected by comparing the relative densities of microbes from the scanning electron micrographs of Fig. 2. A general increase in microbial metabolic activity by amphipod grazing at natural densities has also been established (Morrison & White, 1980). This is reflected in this study by the lowered adenylate energy charge and ATP/adenosine ratio in the surface microbiota not grazed by amphipods. In nature amphipods actively seek out the detritus with the higher content of bacteria as indicated by the "signature" fatty acid content (White *et al.*, 1979d). At this point it is not clear whether we are examining the residual community, the community colonizing the newly created space, or a combination of both.

EFFECTS OF AMPHIPOD GRAZING IN THE LABORATORY AND FIELD

Grazing increased the microeukaryotes, particularly the microfauna, as reflected in the polyenoic fatty acids with alpha linolenic (w3) unsaturation. Grazing in the laboratory increased the total bacterial biomass (muramic acid, wall glucosamine) and indicated a higher total photosynthetic microeukaryote density (lipid galactose). With grazing the diatoms predominated as indicated in the higher content of 20 : 5w3. Diatoms contain very little 18-carbon polyenoic fatty acids and are enriched in 16 : 3w4 and 20 : 5w3 (Darley, 1977; Volkman & Johns, 1977). The metabolic status of the laboratory grazed surface microbiota is higher as reflected in the increased ATP/adenosine ratio. There are, however, more algae (higher 18 : 3w3, and ratio of 22 : 6w3 to 16 : 0) in the surface microbiota grazed in the field.

No measures of protozoal grazing or grazers not retained in the litter baskets in the field were made. In previous studies when amphipod grazing pressure is increased, the activity and biomass of the detrital microbiota reach a maximum and then decrease as grazing pressure exceeds the capacity for recovery (Morrison, 1980).

In the present study, the nutritional status of the amphipods themselves as reflected in the adenylate energy charge and the neutral lipid (triglyceride) glycerol to phospholipid ratio in amphipods from both the laboratory and field were nearly equal. The neutral lipid glycerol to phospholipid ratios of the amphipods from the field and laboratory were 50-fold higher than starved amphipods indicating that the possible stresses of laboratory life on the grazers was not reflected in their metabolic status.

PARTITIONING OF THE DETRITAL MICROBIOTA BETWEEN AMPHIPODS

The apparent differences in mouth parts of the two sympatric amphipods (Fig. 3) are reflected in the scanning electron micrographs of the grazed detrital microbiota (Fig. 2). The suggestive differences in the morphology of the grazed microbiota can be quantitatively defined by the data of Tables I–IV, columns D and E. Grazing by *Melita* with less setosed and greatly articulated mandibles and maxillae, leaves a surface community greatly enriched in bacteria (muramic acid, 18 : 1w7, proportions of a + i 15 : 0/ 15 : 0, 18 : 1w7/ 16 : 0). The total photosynthetic microeukaryote biomass is increased as reflected in the lipid galactose, and this greater biomass shows increased rates of sulfolipid synthesis. The total microbial biomass is increased as reflected in the extractable lipid phosphate and in the higher rate of phospholipid biosynthesis. *Melita* apparently specifically harvests the non-photosynthetic microeukaryotes and the grazed detritus is overgrown by bacteria and diatoms. The selective removal of the microfauna removes a class of microbial grazers which could by itself lead to the larger microbial biomass and total synthetic capacity. *Gammarus* feeds with heavily setosed and less articulated mandibles and maxilla (Bousfield, 1973) on bacteria and microeukaryotes with much less specificity. This results in a cleaned detrital surface (Fig. 2, Morrison & White, 1980). More of the microeukaryotes remain, as reflected in the higher total lipid glycerol and the higher ratio of lipid glycerol to lipid phosphate. The grazed surface shows intense metabolic activity reflected in the higher total adenosine nucleotides (with lowered total biomass), higher ATP, and higher adenylate energy charge. The ratio of phospholipid synthesis to total phospholipid and sulfolipid synthesis to lipid galactose in the *Gammarus* grazed detritus is about half that of the *Melita* grazed communities. The more generalized grazing of *Gammarus* produces a smaller, more active residual microbiota with smaller specific synthetic capacity.

This study gives a clear indication that to reproduce the detrital microbial biomass, metabolic activity, nutritional status and community structure found in detrital samples taken from nature, as in microcosm experiments used for testing biodeterioration rates, the grazing of amphipods at natural population densities must be included. It also documents that sympatric amphipod grazers finely partition the surface microbiota.

ACKNOWLEDGEMENTS

This work was supported by grant OCE 76-19671 from the Biological Oceanography Section of the National Science Foundation; grant 04-7-158-4406 from the National Oceanic and Atmospheric Administration, Office of Sea Grant, Department of Commerce; and grants R-0806143010 and R-0806143020 from the U.S. Environmental Protection Agency.

Scanning electron micrographs were prepared by W. I. Miller III, Department of Biological Science, Florida State University. The advice of D. Thistle of the Department of Oceanography, Florida State University is gratefully acknowledged.

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